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ORIGINAL REPORT

Risk of Relapse of Childhood Acute Lymphoblastic Leukemia Is Predicted By Flow Cytometric Measurement of Residual Disease on Day 15 Bone Marrow

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See accompanying editorial on page 5121 and articles on pages 5175 and 5189

A B S T R A C T

Purpose

Speed of blast clearance is an indicator of outcome in childhood acute lymphoblastic leukemia (ALL). Availability of measurement of minimal residual disease (MRD) at an early time point with a reduced-cost method is of clinical relevance. In the AIEOP-BFM-ALL (Associazione Italiana Ematologia Oncologia Pediatrica and Berlin-Frankfurt-Münster Study Group) 2000 trial, patients were stratified by levels of polymerase chain reaction (PCR) MRD at day +33 and +78. AIEOP studied the prognostic impact of MRD measured by flow cytometry (FCM) at day 15 of induction therapy.

Patients and Methods

Bone marrow samples from 830 Italian patients were collected on day 15, after 14 days of steroids, and one dose of intrathecal methotrexate, vincristine, daunorubicine, and asparaginase. Cells were analyzed by four-color FCM for detection of leukemia-associated immunophenotypes.

Results

Three patient risk groups were identified by FCM: standard (< 0.1% blast cells; 42% of the total), intermediate (0.1 to < 10%; 47%), and high (≥ 10%; 11%). Their 5-year cumulative incidences of relapse were 7.5% (SE, 1.5), 17.5% (SE, 2.1), and 47.2% (SE, 5.9), respectively. In multivariate analysis, FCM was the most important prognostic factor among those available by day 15, with two-fold and five-fold increase in the risk of relapse compared with patients with less than 0.1%. PCR MRD, when added to the model, had significant prognostic impact; yet high levels of FCM MRD retained an independent ability to detect a significantly higher risk of relapse.

Conclusion

Measurement of FCM MRD in day 15 bone marrow was the most powerful early predictor of relapse, applicable to virtually all patients; it may complement PCR MRD–based stratification including later time points, thus allowing additional treatment tailoring.

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The Appendix is included in the full-text version of this article, available online at www.jco.org. It is not included in the PDF version (via Adobe® Reader®).

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INTRODUCTION

Recent advances in the treatment of childhood acute lymphoblastic leukemia (ALL) allow the vast majority of patients to achieve complete remission and then to be cured. Nevertheless, relapse represents the main cause for treatment failure.[1] Early response to therapy is a primary prognostic factor.[1,2] Blast count in the peripheral blood on day 8, or in the bone marrow on day 15, have been widely used to deliver risk-directed therapy.[3-5] Recently, most groups explored the feasibility of measurement of submicroscopic levels of leukemic cells, minimal residual disease (MRD). During the last 10 years, levels of MRD proved to be able to predict patient's prognosis, regardless of traditional presenting features, such as age and leukocyte count,[6-9] and are now widely applied to assign patients to different risk categories during first-line treatment. In the recent AIEOP-BFM-ALL (Associazione Italiana Ematologia Oncologia Pediatrica and Berlin-Frankfurt-Münster Study Group) 2000 study, patients were stratified into three risk groups according to MRD results as detected by real-time quantitative polymerase chain reaction (RQ-PCR) of receptor gene rearrangements, in bone marrow

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samples collected at the end of induction phases IA (day 33), and IB (day 78).[10,11]

Molecular detection of MRD has been well standardized.[12] Although less standardized, flow cytometry (FCM) is faster, generally less expensive, and provides information in a higher percentage of patients than molecular methods.[13,14] Although it was initially applied mainly in single-institution settings,[15-18] in a recent Children's Oncology Group study more than 2,000 patients from more than 100 centers were investigated with this technique.[19]

As part of the recently closed AIEOP-BFM 2000 trial, selected Italian institutions submitted day 15 bone marrow samples to a single reference laboratory for determination of MRD by FCM. We demonstrate that measurement of FCM MRD at one single, early time point in day 15 bone marrow is feasible and has a robust prognostic impact.

PATIENTS AND METHODS

Study Population

Between December 2000 and December 2004, 1,476 consecutive children, age 1 to younger than 18 years, with newly diagnosed ALL, were enrolled in the AIEOP-BFM-ALL 2000 study (NCT00613457) by 39 Italian centers which had it approved by their institutional review boards.[10,11] Of these patients, 1,252 were recruited in 34 centers, which participated in a research project on FCM MRD, requiring a bone marrow aspirate on day 15, in addition to aspirates on day 33 and 78, mandatory for PCR MRD–based patient stratification.[20] Overall, samples for FCM MRD were available for 830 patients; main analyses are performed on 815 patients, accounting for 66.5% of the 1,226 non–Philadelphia-positive patients in the cohort. Informed consent to participate in the study was obtained for all patients by parents or legal guardians.

Diagnostic Studies

Bone marrow samples were shipped overnight to the AIEOP reference laboratory in Padua. The diagnosis of ALL was based on standard morphologic, cytochemical, immunophenotypic, and genetic analyses.

Bone marrow aspirate was examined on day 33 (time point 1 [TP1]) to assess complete remission (CR), defined as no physical signs of leukemia, bone marrow with active hematopoiesis, and fewer than 5% leukemic blast cells, normal CSF. Patients who failed to achieve CR were assigned to high-risk group; those who did not achieve CR after phase IB and consolidation were defined resistant and counted as treatment failures.

Leukemia-associated immunophenotypes were studied at diagnosis by using the following monoclonal fluorochrome-conjugated antibody combinations: CD58/CD10/CD19/CD34; CD20/CD10/CD19/CD34; CD10/CD34/CD19/CD45; CD10/CD11a/CD19/CD45 for B-cell precursor ALL; and CD99/CD7/CD5/sCD3; CD99/CD7/sCD3/cyCD3; TdT/CD7/sCD3/cyCD3 for T-ALL.[21-23] At least two selected combinations were applied during follow-up for MRD detection.

PCR-MRD evaluation at TP1 and at day 78 (TP2) was performed in the reference laboratory in Monza.[20,24]

Definition of Risk Groups

The high-risk group included patients with any of the following criteria: t(4;11) or MLL/AF4; prednisone poor response ($\geq$ 1,000 blasts/μL on day 8 peripheral blood after 7 days of prednisone and one dose of intrathecal methotrexate on day 1)[5]; inability to achieve CR on TP1; high burden ($\geq 10^{-3}$) of PCR-MRD at TP2. The standard-risk group included patients who lacked high-risk criteria and tested negative by PCR-MRD performed by using two sensitive markers ($\geq 1 \times 10^{-4}$) at both TP1 and TP2.[20] The intermediate-risk group included the remaining patients, and those not evaluated by PCR MRD.

Treatment

After 7-day monotherapy with prednisone and one dose of intrathecal methotrexate, treatment was complemented with corticosteroid (randomized between prednisone and dexamethasone), vincristine, daunorubicin, and L-asparaginase (Appendix Table A1, online only). Remission induction was followed by element IB with intravenous cyclophosphamide and cytarabine, intrathecal methotrexate, and oral mercaptopurine. Allogeneic stem cell transplantation was indicated for a subgroup of patients at very high risk.

Statistical Analysis

Event-free survival (EFS) curves were estimated by the Kaplan-Meier method, with Greenwood SEs and compared according to the log-rank test. The starting point for the observation time was date of diagnosis. Death in induction, resistance, relapse, death in continuous CR, or secondary malignant neoplasm were considered as events in the calculation of EFS probability (every patient is alive, by definition of the study population, from diagnosis up to day 15). Cumulative incidence curves of relapse were estimated adjusting for competing risks of the other events and were compared with the Gray's test. In both analyses, the observation time was censored at the last follow-up date if no event was observed. The Cox regression model on the cause specific hazard of relapse was applied to study the prognostic value of day 15 FCM MRD both after adjusting for candidate prognostic factors available by day 15 (age and leukocyte count, as summarized by National Cancer Institute criteria, immunophenotype, TEL/AML1 status, response to prednisone, and morphological evaluation of day 15 bone marrow) and after adjusting also for PCR MRD stratification. Results of the full models are shown with estimated hazards ratios (HR), 95% CIs, and *P* values (according to the Wald test). The likelihood ratio test was applied to test the interaction between FCM MRD and

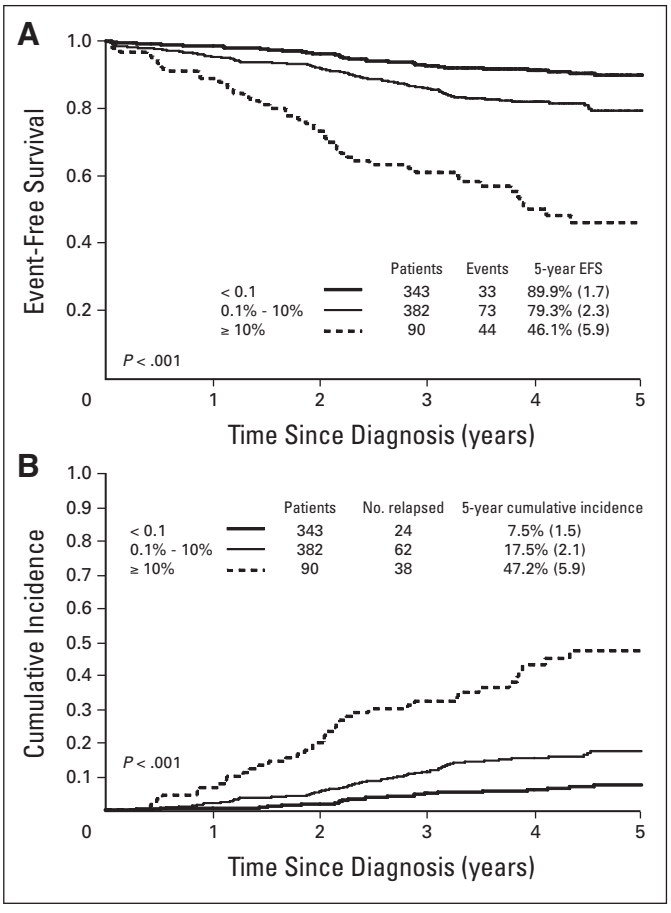

Fig 1. (A) Event-free survival (EFS) and (B) cumulative incidence of relapse in 815 patients treated in the AIEOP-BFM-ALL 2000 trial, stratified into three risk groups according to minimal residual disease on day 15 marrow as detected by flow cytometry. NOTE. 5-year event-free survival and cumulative incidence presented as percentage and standard error.

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PCR MRD. A graphical check on each regressor did not detect major departures from proportional hazard assumption. Follow-up was updated as of February 29, 2008, with a minimum potential follow-up time of 3 years for every patient.

RESULTS

The 815 patients with childhood ALL (non–Philadelphia positive) studied for FCM MRD on day 15 bone marrow were comparable to those for whom a day 15 bone marrow sample was not submitted to the reference laboratory (Appendix Table A2, online only), and had a similar 5-year cumulative incidence of relapse: 16.4% (SE, 1.4) and 16.9% (SE, 1.9), respectively ($P = .68$).

Of the 815 patients, 124 relapsed (15.2%) in the bone marrow isolated (n = 80) or combined (n = 10), CNS (n = 12), testis (n = 15), and other sites (n = 7). Other treatment failures were resistance (n = 1), death in induction (n = 10), death in remission (n = 11), and second malignancies (n = 4).

Prognostic Relevance of Day 15 FCM MRD

Of the 815 patients, 187 (22.9%) tested negative (< 0.01%), whereas the remaining were distributed among different levels of positivity: 156 (19.1%) had 0.01% to < 0.1%; 213 (26.1%) had 0.1% to < 1%; 169 (20.7%) had 1% to < 10%; and 90 (11.0%) had at least 10% of blast cells. With a median follow-up of 4.6 years, their 5-year EFS was 91.5% (SE, 2.1), 88.1% (SE, 2.8), 83.5% (SE, 2.8), 74.2% (SE, 3.6), and 46.1% (SE, 5.9), respectively. Their estimated 5-year cumulative incidence of relapse was significantly different ($P < .001$): 6.4% (SE, 1.9) in the negative FCM MRD patients, and 8.7% (SE, 2.4), 12.8% (SE, 2.5), 23.4% (SE, 3.5), 47.2% (SE, 5.9) in categories with increasing positivity (Appendix Fig A1, online only).

From a clinical standpoint, it may be relevant to identify early during initial therapy the two subgroups of patients with significantly different outcome (ie, at lowest or highest risk of leukemia relapse). To this purpose, our FCM MRD data suggest appropriate cut-offs of 0.1% for standard risk, and more than 10% for the high-risk group. This results in three risk groups with significantly different probabilities of EFS and cumulative incidence of relapse according to day 15 FCM MRD ($P < .001$; Fig 1).

Comparison Between Morphology and FCM MRD Analysis on Day 15 Bone Marrow

Morphological evaluation of day 15 bone marrow smear was performed at the treating center in 695 patients (85.3%). Of those patients, 503 (72.4%) were defined M1 (blast cells < 5%), while 133 (19.1%) were classified as M2 (5% to less than 25%), and 59 (8.5%) were classified as M3 (≥ 25%); these subgroups had a cumulative incidence of relapse of 11.1% (1.5), 21.2% (3.8), and 36.8% (6.5; Table 1). Seven patients who had lower than 0.1% MRD by FCM were classified by morphology as M3 in the treating centers. Of note, six of these patients classified PCR-MRD negative at day 33, one was not assessable.

Among 503 M1 patients, FCM MRD was able to discriminate prognosis; 247 patients with FCM MRD lower than 0.1% had 13 relapses (5.3%), and the rate progressively increased to reach 36.8% in the 19 patients with FCM MRD ≥10%. There was a significant difference in the cumulative incidence of relapse ($P < .001$; data not shown).

Comparison With AIEOP-BFM-ALL 2000 PCR-Based Stratification

The value of our FCM MRD derived stratification in discriminating prognosis within a stratification based on PCR MRD was assessed

Table 1. Distribution of Leukemia Relapses in 815 Patients Enrolled Onto the AIEOP-BFM-ALL 2000 Trial According to Levels of Residual Disease Assessed by Flow Cytometry on Day 15 Marrow, and to Bone Marrow Morphology on Day 15 or PCR on Day 33 and 78

	FCM-MRD Risk Group												5-Year Cumulative Incidence of Relapse	
	Standard (< 0.1%)			Intermediate (0.1 to < 10%)			High (≥ 10%)			Total				
Parameter	No. of Patients	No. of Patients Relapsed	%	No. of Patients	No. of Patients Relapsed	%	No. of Patients	No. of Patients Relapsed	%	No. of Patients	No. of Patients Relapsed	%	%	SE
Morphology														
M1	247	13	5.3	237	31	13.1	19	7	36.8	503	51	10.1	11.1	1.5
M2	41	6	14.6	70	12	17.1	22	8	36.4	133	26	19.5	21.1	3.8
M3	7	2	28.6	17	5	29.4	35	14	40.0	59	21	35.6	36.8	6.5
Not known	48	3	6.3	58	14	24.1	14	9	64.3	120	26	21.7	23.6	4.1
Total	343	24	7.0	382	62	16.2	90	38	42.2	815	124	15.2		
PCR-MRD														
SR*	153	7	4.6	94	5	5.3	3	0		250	12	4.8	5.0	1.4
IR	103	11	10.7	176	41	23.3	52	23	44.2	331	75	22.7	25.5	2.7
HR	4	1	25.0	23	6	26.1	25	13	52.0	52	20	38.5	38.6	6.8
Not known	83	5	6.0	89	10	11.2	10	2	20.0	182	17	9.3	10.7	2.5
Total	343	24	7.0	382	62	16.2	90	38	42.2	815	124	15.2		
5-year cumulative incidence of relapse, % (SE)		7.5 (1.5)			17.5 (2.1)			47.2 (5.9)						

Abbreviations: PCR, polymerase chain reaction; FCM, flow cytometry; MRD, minimal residual disease; SE, standard error; SR, standard risk; IR, intermediate risk; HR, high risk.
*PCR-MRD negative at both time points 1 and 2, performed with at least two sensitive (≥ 10^{-4}) molecular markers.[19]

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by analyzing 633 patients (77.7%) with available data by both techniques. PCR MRD groups defined at day 33 and 78 had a rather heterogeneous composition with regard to FCM MRD at day 15. The 5-year cumulative incidence of relapse between classes defined according to FCM or PCR was similar: standard risk of 7.5% (SE, 1.5) versus 5.0% (1.4); intermediate risk of 17.5% (SE, 2.1) versus 25.5% (SE, 2.7); high risk of 47.2% (SE, 5.9) versus 38.6% (SE, 6.8), respectively (Table 1). Patients classified by PCR MRD as standard risk or high risk had a very good or poor prognosis independently of FCM MRD results. Conversely among patients at intermediate risk by PCR MRD, day 15 FCM MRD levels discriminated subgroups with different cumulative incidences of relapse: 10.8% (SE, 3.3), 26.3% (SE, 3.8), and 55.7% (SE, 8.7), respectively. Due to small numbers, a formal test for interaction between PCR MRD and FCM MRD levels did not reach statistical significance, even when limited to the two strata of intermediate risk and high risk PCR MRD patients ($P = .39$).

FCM MRD by Presenting Features

In order to assess the predictive value of FCM MRD across conventional prognostic factors, we evaluated the incidence of relapse in both B- and T-lineage patients categorized as National Cancer Institute standard (age 1 to 9 years and leukocyte count lower than 50,000/μL) and high risk (others) groups subdivided according to the levels of day 15 FCM MRD. As illustrated in Figure 2, of 566 National Cancer Institute standard-risk patients, 51 had $\geq$ 10% of blasts and they had a 5-year cumulative incidence of relapse of 43.1% (SE, 8.6); of 249 National Cancer Institute high-risk patients, 92 had lower than 0.1% of blasts with a 5-year cumulative incidence of relapse of 5.4% (SE, 2.4).

Day 15 FCM MRD maintained a significant prognostic impact on the incidence of relapse also when 724 B-cell precursor ALL patients were analyzed separately ($P < .001$). Among 87 patients with T-ALL, FCM was able to identify subsets at different prognosis; in particular, 50% of the relapses occurred in the 20% of patients with more than 10% blasts (Fig 3).

The prognostic value was evident also within categories of patients with other widely used prognostic factors. The two subgroups of patients defined by prednisone response on day 8 were further subdivided by levels of FCM MRD in subgroups with significantly different outcome: the 5-year cumulative incidence of relapse was 8.5% (SE, 1.9), 19.5% (SE, 2.6), and 44.2% (SE, 6.2) in prednisone good responders with FCM MRD levels of lower than 0.1%, 0.1% to 10%, or higher than 10%, respectively ($P < .001$); for poor responder patients, the incidences of relapse were 6.3% (SE, 3.6), 11.2% (SE, 3.9), and 66.7% (SE, 19.2), respectively ($P < .001$). The risk of relapse in patients with TEL/AML rearrangement is illustrated in Figure 4.

Multivariate Cox regression model on the hazard of relapse was first performed for investigating the role of FCM MRD on top of the most important prognostic variables which are routinely available by day 15 from start treatment. In this analysis (Table 2, model I), FCM MRD was the most important prognostic factor: intermediate and high levels were related approximately to a two-fold and five-fold increase in the risk of relapse, as compared with patients with low levels. PCR MRD, measured on day 33 and 78 marrow, when added to the model, had a significant prognostic impact (model II); yet high levels of FCM MRD did retain significance with respect to the risk of relapse even after adjusting for PCR MRD.

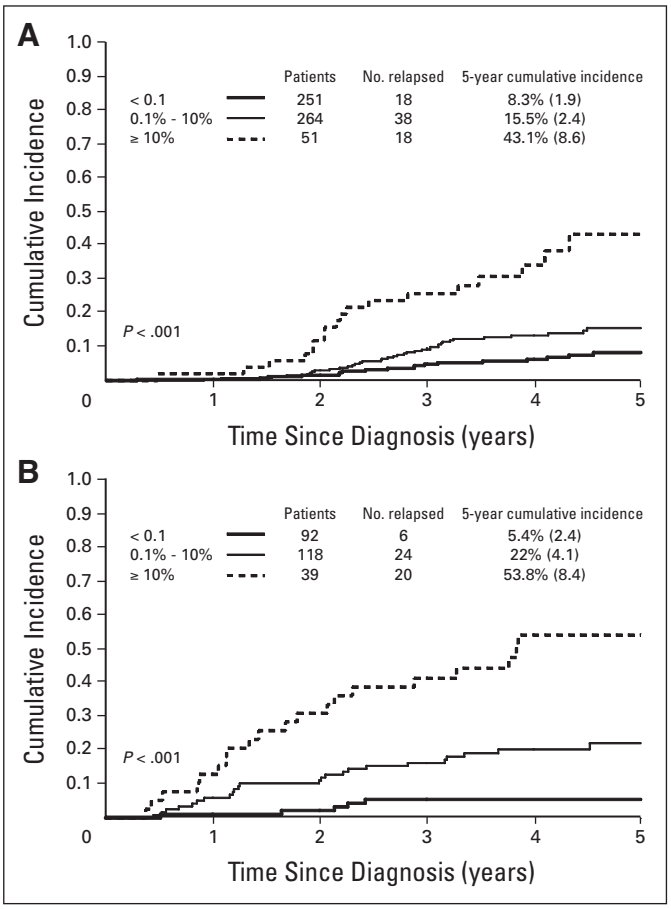

Fig 2. Prognostic impact of minimal residual disease on day 15 marrow as detected by flow cytometry in patients treated in the AIEOP-BFM-ALL 2000 trial and stratified according to National Cancer Institute criteria: (A) standard risk or (B) high risk. NOTE. 5-year cumulative incidence presented as percentage and standard error.

DISCUSSION

At the outstanding level of cure achieved by contemporary chemotherapy trials, early discrimination of patients at different prognosis provides leukemia therapists the opportunity to tailor the treatment schedule, not only to intensify the therapeutic approach for patients at higher risk for leukemia relapse, but also to prevent a significant proportion of patients from undue exposure to more toxic treatment elements, when cure can be likely achieved with less intensive therapy. Measurement of blast clearance allowing quantitative definition of MRD becomes a mandatory tool for childhood ALL therapy. In vivo response to prednisone originally described by the BFM group[5] has been widely used to this purpose; although it is cheap and universally accessible, its further use is hampered by limited sensitivity and specificity at the current level of cure rate. Thus, sophisticated and expensive technologies have been approached and developed during the last decade. PCR-based method has been applied by several groups in studies which documented a high level of sensitivity, standardization, and accordingly, applicability.[1,6-9,11] In the year 2000, AIEOP and BFM groups started a new ALL study with patients stratified by PCR MRD.

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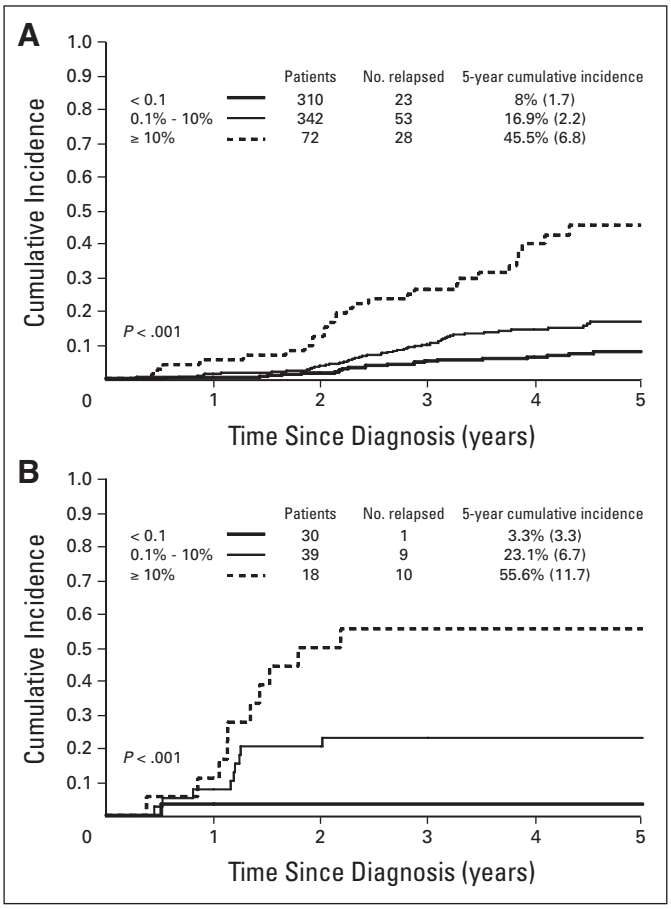

Fig 3. Prognostic impact of minimal residual disease on day 15 marrow as detected by flow cytometry in patients treated in the AIEOP-BFM-ALL 2000 trial and stratified according to immunophenotype: (A) B-cell precursor acute lymphoblastic leukemia (ALL) or (B) T-ALL. NOTE. 5-year cumulative incidence presented as percentage and standard error.

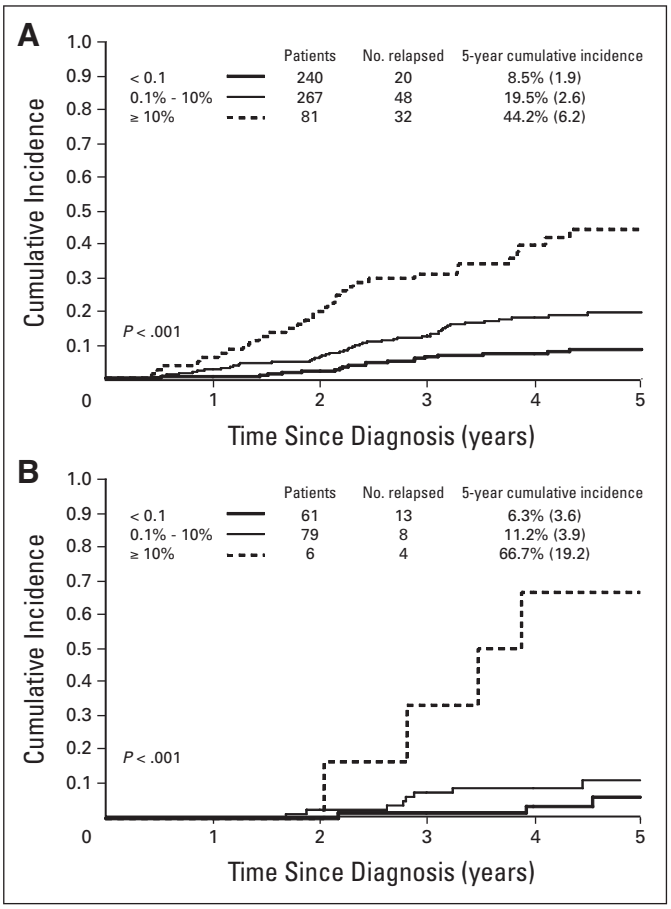

Fig 4. Prognostic impact of minimal residual disease on day 15 marrow as detected by flow cytometry in patients treated in the AIEOP-BFM-ALL 2000 trial and stratified according to TEL/AML1: (A) negative or (B) positive. NOTE. 5-year cumulative incidence presented as percentage and standard error.

The use of FCM MRD, although formerly less standardized, may represent an important additional technology, due to its fast and cheaper availability. A significant note of caution has been represented by the fact that most of the initial reports on FCM MRD application in childhood ALL derived from single centers with documented skills.[15-17] In this context, in the AIEOP-BFM-ALL 2000 study FCM MRD was also measured in a large fraction of patients at day 15 to evaluate its prognostic impact. The participation in this prospective study was not mandatory. The reproducibility of our approach has been validated in a multicenter setting by four independent laboratories, demonstrating its feasibility and robustness by a very high concordance rate at day 15 and 78, and a lower at day 33 at the end of induction.[25]

We found that approximately 40% of patients with childhood ALL treated with contemporary BFM-based chemotherapy achieve a subtotal clearance of leukemic cells ($<$ 0.1% among bone marrow nucleated cells) after exposure to 14 days of steroids and one dose of intrathecal methotrexate, intravenous vincristine, anthracycline, and asparaginase. These patients have an excellent treatment outcome with more than 90% of them remaining free from relapse after 5 years. Conversely, the minority of patients with insufficient response ($\geq$ 10% blast cells) by day 15 had an unfavorable outcome due to excess of leukemia relapse. These findings highlight the prognostic importance of early blast clearance kinetics.

Coustan-Smith et al at St Jude Children's Research Hospital (Memphis, TN)[15] studied FCM MRD at a later time point (day 19), in 248 patients and found that 46% were MRD negative ($<$ 0.01%). At this time point, the authors could segregate by FCM MRD two risk groups of almost equal size but with significantly different outcome, especially those at nonhigh risk. More recently, the Children's Oncology Group reported a large study of FCM MRD on the peripheral blood at day 8, end induction (day 29), and end consolidation marrows, in 2,143 children with B-cell precursor ALL. The presence of MRD in day 8 blood and day 29 marrow was associated with a lower EFS in all risk groups.[19] In this study around 30% of all patients were MRD negative ($<$0.01%) and had a very good outcome. Measurement of FCM MRD at the end of induction (day 29) showed that a very high proportion of patients (more than 80%) were MRD negative and thus at a very good prognosis. Overall, the two studies are very well in keeping and thus our findings confirm and extend those observations.

We have documented that FCM MRD testing on day 15 marrow may herald a low incidence of leukemia relapse (7.5%) in a large proportion of patients (42%), or otherwise a 47.2% incidence in a small subgroup of 11% of patients with FCM MRD $\geq$ 10%. In particular, it turned to be in our setting the most potent, independent

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Table 2. Multivariate Analysis of the Prognostic Impact on Hazard of Relapse of Different Factors, in Particular of MRD as Measured by Flow Cytometry at Day +15 and MRD as Measured by PCR at Days +33 and +78 (493 patients with known data on all covariates in the two models)

Parameter	Model I			Model II		
	Hazard Ratio	95% CI	*P*	Hazard Ratio	95% CI	*P*
Flow cytometry MRD on day 15 marrow						
Standard risk, < 0.1%	1			1		
Intermediate risk, 0.1 to < 10%	1.87	1.05 to 3.34	.03	1.43	0.79 to 2.59	.24
High risk, ≥ 10%	4.91	2.35 to 10.27	< .001	2.86	1.30 to 6.27	.009
Morphological blast count on day 15 marrow						
M1	1			1		
M2	1.52	0.88 to 2.61	.13	1.47	0.85 to 2.53	.16
M3	1.99	0.96 to 4.13	.07	2.03	0.93 to 4.39	.07
Response to prednisone						
Good	1			1		
Poor	0.60	0.27 to 1.31	.20	0.61	0.28 to 1.34	.22
National Cancer Institute criteria						
Standard	1			1		
High	1.49	0.91 to 2.46	.11	1.56	0.94 to 2.59	.09
Immunophenotype						
B-cell precursor ALL	1			1		
T-ALL	1.49	0.76 to 2.93	.25	1.33	0.67 to 2.64	.42
TEL/AML1						
Positive	1			1		
Negative	1.24	0.65 to 2.34	.52	0.97	0.50 to 1.87	.92
PCR-MRD on days 33 and 78 marrow						
Standard risk	—	—	—	1		
Intermediate risk	—	—	—	3.59	1.77 to 7.30	< .001
High risk	—	—	—	3.99	1.56 to 10.20	.004

Abbreviations: MRD, minimal residual disease; PCR, polymerase chain reaction; ALL, acute lymphoblastic leukemia.

prognostic factor among those available by day 15, including National Cancer Institute criteria and prednisone response. Furthermore, ≥ 10% FCM MRD was an independent risk factor for leukemia relapse even when PCR MRD stratification was included in this multivariate analysis.

The morphological evaluation of day 15 marrow is often used to assess early response and risk group assignment. Our FCM MRD data suggest that overall there was a good correlation between FCM and morphology, but that there were discrepant cases. In particular, we documented that some patients classified as M1 had more than 10% blasts at flow cytometry MRD evaluation and their risk of relapse was of 36.8%. On the contrary, we observed seven patients who were M3, in whom FCM showed a result less than 0.1%: of those patients, only two relapsed while five remain disease free. Thus, morphology data should be taken with caution, particularly if the marrow is hypocellular and evaluation is not centralized.

In conclusion, measurement of residual leukemia by FCM on day 15 bone marrow was applicable in a centralized reference laboratory to the large majority of patients, and provided results within few hours. The subgroup of patients with high FCM MRD at day 15 fared especially poorly independent of all clinical and biologic features. This information may be used to refine stratification of patients by PCR MRD, with FCM addressing more the speed of response, and the latter the quality of remission. The cost of FCM MRD is widely affordable in most settings, even outside the cooperative groups in which most PCR-based studies have been developed.[12] File transfer for second opinion in the frame of interinstitutional cooperation was also shown to be feasible.[26] The prognostic value of PCR MRD remains undisputed. Yet, we think that the two methods may be combined in a strategy aimed at defining the risk of failure of all patients with childhood ALL. In our next AIEOP-BFM-ALL trial, based on PCR MRD stratification, we will use FCM MRD for identification of 30% of patients with excellent outcome even before the end of induction; they will be randomly assigned for reduction of anthracycline dose during induction, to minimize treatment-related fatalities. The contribution of PCR MRD could be pivotal also for identification of additional patients at high risk. The choice of methodology for measurement of MRD in childhood ALL largely depends on the aims of the study, the resources available, and the treatment strategy.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

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